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# Mathematical modeling of *Lactobacillus viridescens* growth in vacuum packed sliced ham under non isothermal conditions

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**Abstract**

Lactic acid bacteria (LAB) are responsible for the spoilage of vacuum packed meat products, as ham. Temperature is the main factor affecting the microbial dynamics and its variation during the production, distribution and storage of foods is considerable. Thus, the use of mathematical models to describe the microbial behavior under variable temperatures can be very useful in predicting the food shelf life. This study evaluated the growth of *Lactobacillus viridescens* in sliced ham under non isothermal conditions, and assessed the predictive ability of the Baranyi and Roberts model using parameters obtained isothermally in culture medium (MRS). To obtain the BAL growth, the fresh ham piece was sterilized, sliced, inoculated with bacteria and stored in a temperature-controlled incubator. For the establishment of the secondary models, the primary model parameters were obtained isothermally in the culture medium at 4, 8, 12, 16, 20 and 30 ° C, in which there was no lag phase observed; the square root model was selected to describe the dependence of the $\mu_{max}$ parameter (maximum specific growth rate) with the temperature, and the $y_{max}$ parameter (maximum population) was represented by an average because there was no significant influence of the temperature. The mathematical models were validated with *L. viridescens* growth data in ham under five variable temperature conditions (NI-1 (4-8-12-16 °C), NI-2 (12-16-20-25 °C), NI-3 (25-20-16-12-8-4 °C), NI-4 (16-12-8-4 °C) and NI-5 (12-8-4-8-12 °C)), and its predictive ability were assessed through statistical indexes (bias factor, accuracy factor and RMSE), with good results (bias factor between 0.9450 and 1.0326; accuracy factor between 1.0382 and 1.0682, and RMSE between 0.7641 and 1.3317), especially in increasing temperature, where the prediction was safe. The validated model can be used to estimate the shelf life of a commercial ham under different temperature conditions.



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*Keywords:* lactic acid bacteria; dynamic modeling; meat products

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## 1. Introduction

The color of cured meats is one of the most important factors affecting consumer acceptability. Green discoloration in cured meats is a periodic problem for the meat industry and is usually caused by particular microorganisms which are able to produce oxidizing substances that act on the cured meat pigments[1]. *Lactobacillus viridescens* has been described as the organism frequently responsible for microbial greening in cured sausage and ham products [2,3,4].

The temperature has great influence on the kinetics of microbial growth, especially for chilled foods, when it usually varies greatly during transport, retail and at home[5]. Due to this, the mathematical modeling of the microbial growth is directed to obtaining models describing non-isothermal environment and make it possible to predict the shelf -life of foods in real conditions[6,7].

The most common method for validating models using new data is to carry out experiments directly on the food product of concern[8]. The objective of this study was to assess the predictive ability of a mathematical model to describe the growth of *L. viridescens* under non isothermal conditions, in order to simulate a real environment this products are subjected, using parameters obtained in culture medium at constant temperatures.

## 2. Materials and methods

### *2.1. Microorganism, inoculum, sample preparation, growth conditions and sampling*

The *L. viridescens* (CCT 5843 ATCC 12706, Lote 22.07) used in this study was purchased in lyophilized form from the collection of cultures of the *André Tosello* Foundation of Tropical Cultures (Campinas, Brazil). The strains were rehydrated, grown in Man, Rogosa and Sharpe (MRS) - *Lactobacillus* medium (Acumedia Manufactures, Michigan, USA), and stored in Eppendorf tubes with MRS containing 20 % glycerol at −24 ºC until its use.

The reactivation of the culture for preparing the inoculum was carried out in MRS at 30 ºC for 18 h, when the concentration of $10^9$ CFU/g is obtained. Then, successive dilutions were performed in test tubes containing MRS until the concentration of 6 x $10^4$ CFU/g.

In order to eliminate the natural bacterial flora, the ham was superficially sterilized with alcohol 70 % (v/v) and sliced in laminar flow chamber. The slices (about 20 g) were inoculated with 1 mL of inoculum and packed into a sterile mixer bag and then in a vacuum plastic bag. The samples were stored in a temperature-controlled incubator (Dist, Florianópolis, Brasil).

The growth of *L. viridescens* in vacuum-packed sliced ham was evaluated in five non isothermal conditions. The programmed temperature profiles are shown in Table 1. The temperature of the incubator was recorded in data logger (Testo174, Lenzkirch, Germany) in every five minutes. In pre-determined time intervals, two samples (duplicate) were taken to determine the *L. viridescens* cells concentration in ham by plate count method. The results were expressed as log (*N*), where *N* is the LAB concentration [CFU/g] at time *t*[h].

Table 1 – Non isothermal temperature profiles designed to assess the growth of *L. viridescens* in ham with the plateaus of temperature (T, in ºC) and time to temperature shift ($t_{shift}$, in hours).

| Profile | $T_1$ [$t_{shift1}$] | $T_2$ [$t_{shift2}$] | $T_3$ [$t_{shift3}$] | $T_4$ [$t_{shift4}$] | $T_5$ [$t_{shift5}$] | $T_6$ [$t_{shift6}$] |
|---|---|---|---|---|---|---|
| NI-1 | 4 [63.0] | 8 [91.6] | 12 [105.0] | 16 [168.0] | | |
| NI-2 | 12 [20.1] | 16 [32.0] | 20 [39.8] | 25 [60.0] | | |
| NI-3 | 25 [4.3] | 20 [10.8] | 16 [20.7] | 12 [37.5] | 8 [71.6] | 4 [168.0] |
| NI-4 | 16 [11.9] | 12 [32.0] | 8 [72.9] | 4 [192.0] | | |
| NI-5 | 12 [16.7] | 8 [50.9] | 4 [155.5] | 8 [189.7] | 12 [248.0] | |

### *2.3 Mathematical modeling and statistical analysis*

The predictions of the microbial growth under non-isothermal conditions were carried out using the Baranyi and Roberts[9] model in a differential form, Eq. (1) and (2), with the initial conditions $\ln(N(0)) = \ln(N_0)$ and $\ln(Q(0)) =$

$\ln(Q_0)$. In these equations, $Q$ is related to the physiological state of the cells [dimensionless], $\mu$ is the maximum specific growth rate [1/h], $N_{max}$ is the maximum population [CFU/g]. The secondary models used in this study were obtained through isothermal growth data of *L. viridescens* in MRS[16]. The square root model[10], Eq. (3), was used to describe the influence of temperature on the maximum specific growth rate (μ). In Eq. (3), $T_{min}$ [°C] is the theoretical temperature for the minimal microbial growth, and $b$ [$h^{-0.5}$ $°C^{-1}$] is an empirical parameter. The parameters $b$ = 0.029 and $T_{min}$ = -1.3 were obtained previously in our laboratory ($R^2$ = 0.9927). No considerable influence of temperature was observed to the maximum population, and thus, the arithmetic average of the values obtained isothermally in MRS was used ($y_{max} = \ln(N_{max}) = 21.0$). There was no lag phase in MRS.

$$\frac{d\left(\ln\left(N(t)\right)\right)}{dt} = \mu\left[\frac{1}{1+\exp\left(-Q(t)\right)}\right]\left[1-\exp\left(\ln\left(N(t)\right)-\ln\left(N_{\max}\right)\right)\right] \quad (1)$$

$$\frac{d\left(Q(t)\right)}{dt} = \mu \quad (2)$$

$$\sqrt{\mu} = b\left(T - T_{\min}\right) \quad (3)$$

The resolution of the differential equations were carried out with Matlab software 7 (MathWorks, Natick, USA) using a Runge-Kutta method (ode23 function). The temperature profile used in the predictions was acquired by data recorded in data logger. The statistical indices *RMSE*, *bias* and *accuracy* factors[11] were used to compare the responses obtained experimentally and the responses predicted by the models.

## 3. Results and Discussion

The experimental data and the predicted growth curves of *L. viridescens* growth under non isothermal conditions are shown in Fig. 1. In the five model predictions, the bias factor ranged from 0.9450 to 1.0326 and the accuracy factor was < 1.070 indicating that the predictions had a small deviation when compared to the observed values.

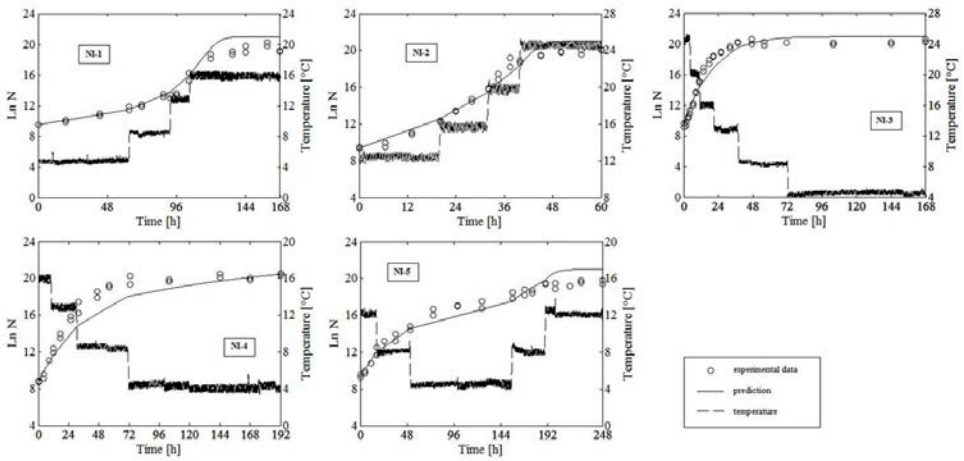


Fig. 1. Prediction of the mathematical model (solid lines) and the experimental data (symbols) of *L. viridescens* growth under non-isothermal conditions (dotted lines).

In increasing temperature conditions (NI-1 and NI-2), the model overestimated the experimental growth (bias factor greater than 1), characterizing a safe prediction. The predictions are in agreement with the observed microbial count until 100 h at NI-1 and until 40 h at NI-2, and there is an overestimation of the stationary phase in both temperature conditions. In decreasing temperature conditions (NI-3, NI-4 and NI-5 (from 0 to 156 h)), the model underestimated the experimental growth (bias factor less than 1), characterizing a dangerous prediction, and there is

no significant overestimation of the stationary phase. These safe and dangerous predictions could be because it is necessary a time for the heat transfer from the incubator atmosphere to the inoculated ham samples, retarding microbial response to that temperature shift; and/or the BAL needed to adapt themselves (metabolic changes) to the new environmental condition. Also, the food matrix may affect the microbial growth because it is more complex than the culture medium[15], and this can be another factor resulting in the deviations between the experimental growth data in ham and the predictions generated by the model developed from growth in MRS.

It is important to note that most deviations were observed near the stationary phase. Many authors[9,12,13] consider that lag and exponential are the growth phases of greatest interest because, in most foods, the spoilage occurs before the stationary phase, as happened in all conditions assessed in this study. For ham and meat products in general, the point for the spoilage caused by lactic acid bacteria is defined as $10^7$ CFU/g[14].

## 4. Conclusion

The model proposed using parameters estimate from isothermal growth data can be used to describe *L. viridescens* growth in refrigerated vacuum packed meat products under non-isothermal conditions and characterize its shelf life due to spoilage. Once the model was developed in culture medium (MRS), the food composition (ham, in this study) can induce deviations from the predictions, more remarkably for decreasing temperature conditions where the predictions were dangerous, therefore it is extremely important to evaluate predictions in all possible conditions in the real scenario.

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